

## **REMARKS**

Claims 1-26 are pending in the application. Claims 1-19, 21-22 and 24-25 are withdrawn as being directed to non-elected inventions. Claims 6-19, 21-22 and 24-25 are canceled herein without prejudice or disclaimer. Claims 20 and 23 are amended herein for clarity and to more particularly define the invention. In addition, withdrawn claims 1-5 are amended herein in order to put them into condition for rejoinder upon allowance of the product claims. Support for these amendments is found in the language of the original claims and throughout the specification, as set forth below. It is believed that no new matter is added by these amendments and their entry and consideration are respectfully requested. In light of these amendments and the following remarks, applicants respectfully request reconsideration of this application and allowance of the pending claims to issue.

### **I. Rejections under 35 U.S.C. § 112, second paragraph**

The Action states that claims 20, 23, and 26 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. Specifically, the Action states that the recitation in claims 20 and 23 of "an miRNA sequence incorporated into the plant miRNA precursor" is confusing because it can be interpreted that the plant precursor has an additional miRNA sequence besides its own endogenous miRNA sequence or it can be interpreted to mean that the endogenous miRNA sequence of said miRNA precursor is replaced by an miRNA sequence. The Action further states that it is unclear whether the recitation "an miRNA sequence" refers to endogenous or exogenous miRNA sequence.

In addition, the Office Action states that claims 20 and 23 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for the recitation of "plant miRNA precursor is modified." Specifically, the Action states that it is unclear if this recitation is referring to modifications anywhere in the miRNA precursor or only in the endogenous miRNA sequence in said precursor.

Claims 20 and 23 as presented herein recite a plant miRNA precursor comprising an exogenous miRNA sequence that replaces an endogenous miRNA sequence and a strand opposite the exogenous miRNA sequence, wherein (a) the exogenous miRNA sequence maintains the length of the endogenous miRNA sequence; and (b) the strand opposite the exogenous miRNA sequence in the isolated plant miRNA precursor is modified to maintain the

secondary structure of the plant miRNA precursor including double strandedness and any mismatches, and further wherein the exogenous miRNA sequence is complementary to and hybridizes with a target nucleotide sequence within said plant, whereby the expression of the target sequence is reduced. Support for this claim language can be found throughout the specification, for example, at least on page 5, lines 11-23; on page 6, lines 1-15; on page 9, lines 7-15 and 23-26; on page 10, lines 1-7; and on page 32, lines 10-20. Thus, no new matter is added by these amendments.

Accordingly, applicants respectfully submit that claims 20 and 23 are now clarified and respectfully request that the rejection of these claims and claims dependent therefrom under 35 U.S.C. §112, second paragraph, be withdrawn.

## **II. Rejections under 35 U.S.C. § 112, first paragraph**

### **A. Enablement**

The Action states that claims 20, 23 and 26 stand rejected under 35 U.S.C. § 112, first paragraph, for allegedly failing to comply with the enablement requirement. Specifically, the Action states that the current claim language can encompass the embodiment wherein an additional miRNA sequence is incorporated into the miRNA precursor rather than replacing the native miRNA precursor with another.

As discussed above, claims 20 and 23 as presented herein recite that an exogenous miRNA sequence replaces the endogenous miRNA sequence. Accordingly, applicants respectfully submit that the rejection of claims 20, 23 and 26 under 35 U.S.C. § 112, first paragraph, has been overcome and respectfully request its withdrawal.

### **B. Written description**

The Action states that claims 20, 23 and 26 stand rejected under 35 U.S.C. § 112, first paragraph, for allegedly failing to comply with the written description requirement. Specifically, the Action states that the specification does not have adequate written description for the genus of plant miRNA precursor sequences comprising native and non-native miRNA sequences. Further, the Action states that the instant specification and originally filed claims appear not to provide support for the phrase "plant miRNA precursor is modified to correspond to the modification made in the miRNA, the modification to the plant miRNA precursor" and

that such a phrase would encompass modifications anywhere within the miRNA precursor sequence and not just within the miRNA sequence.

As discussed above, claims 20 and 23 as presented herein recites that an exogenous miRNA sequence replaces the endogenous miRNA sequence and that the strand opposite the exogenous miRNA sequence in the isolated plant miRNA precursor is modified to maintain the secondary structure of the plant miRNA precursor including double strandedness and any mismatches. These amendments clarify that the exogenous miRNA sequence replaces, and is not in addition to, the endogenous miRNA sequence and that the modifications that are made to the miRNA precursor are made only to the strand opposite the exogenous miRNA sequence.

In view of the amendments and arguments presented herein, applicants respectfully submit that the rejection of claims 20, 23 and 26 under 35 U.S.C. § 112, first paragraph, has been overcome and respectfully request its withdrawal.

#### **V. Rejection under 35 U.S.C. § 103(a).**

The Office Action states that claims 20, 23, and 26 are rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Cullen et al. (U.S. Patent Publication No. 2004/0053411) in view of Llave et al. (*Plant Cell* 14:1605-1619 (2002)) and Rheinhart et al. (*Genes & Development* 16:1616-1626 (2002)). Specifically, the Action states that Cullen et al. teaches designing an artificial miRNA precursor by modifying a naturally occurring miRNA precursor sequence with an exogenous miRNA to a target. The Action further states that Cullen et al. teaches that said modification comprises incorporating an miRNA sequence of interest into said miRNA precursor by substituting stem sequences of its native miRNA to generate miRNAs suitable for use in inhibiting expression of any target gene of interest. Applicants respectfully disagree with the interpretation of Cullen et al.

As an initial point, Cullen et al. has a filing date that is later than the priority date of the present application. However, as indicated in the Office Action, a provisional application was filed in the case of Cullen et al. (U.S. Provisional Application No. 60/377,224; hereinafter "the Cullen provisional") giving it an earlier priority date. Applicants note, however, that the Cullen provisional is not a complete disclosure of everything that is in Cullen et al. For example, while Cullen et al. mentions that plant miRNA precursors exist, the Cullen provisional fails to disclose

any plant miRNAs or miRNA precursors and simply suggests that the invention "provides RNA molecules (miRNAs) functionally equivalent to siRNAs that can be transcribed endogenously in animal and plant cells." (Cullen provisional, page 2, third full paragraph). However, no examples of plant miRNA precursors are provided in the Cullen provisional, only animal miRNA precursors are discussed. Thus, one of ordinary skill in the art would reasonably interpret this to mean that the constructs described in the Cullen provisional based on animal miRNA precursors are intended to function in a plant cell.

As the Office Action mailed October 31, 2006 in the case of the present application acknowledges, "differences exist in the structure and production of miRNA precursors from diverse sources, such as, animals, plants, metazoan etc. The differences include cellular compartmentalization, timing of precursor processing or types of cofactors involved in the processing of miRNA." Thus, miRNA precursors designed using animal miRNA precursors would not be expected to work in plant cells. Accordingly, one of skill in the art would recognize that the Cullen provisional does not teach the use of plant miRNAs and plant miRNA precursors.

Further, contrary to the contention in the present Office Action, Cullen et al. does not teach the present invention of a plant stably transformed with an miRNA precursor construct, said miRNA precursor construct comprising a promoter functional in a plant cell, wherein the promoter is operably linked to a nucleotide sequence encoding an isolated plant miRNA precursor, said plant miRNA precursor comprising an exogenous miRNA sequence that replaces an endogenous miRNA sequence and a strand opposite the exogenous miRNA sequence, wherein (a) the exogenous miRNA sequence maintains the length of the endogenous miRNA sequence; and (b) the strand opposite the exogenous miRNA sequence in the isolated plant miRNA precursor is modified to maintain the secondary structure of the plant miRNA precursor including double strandedness and any mismatches, and further wherein the exogenous miRNA sequence is complementary to and hybridizes with a target nucleotide sequence within said plant, whereby the expression of the target sequence is reduced.

Cullen et al. describes entirely artificial miRNAs and miRNA precursors. Cullen et al. does not describe an exogenous miRNA sequence that replaces an endogenous miRNA sequence, wherein the exogenous miRNA sequence maintains the length of the endogenous

miRNA sequence as taught by the present invention. Further, Cullen et al. fails to teach or suggest that the strand opposite the exogenous miRNA sequence in the isolated plant miRNA precursor is modified to maintain the secondary structure of the plant miRNA precursor including double strandedness and any mismatches, as is claimed in the present invention. Cullen et al. does not teach or suggest that the secondary structure of the native miRNA precursor is to be considered at all in the designing of the artificial miRNA precursor constructs described therein and the examples provided in Cullen et al. clearly illustrate that this is the case.

For instance, Figure 3A of Cullen et al. illustrates the designed miRNA of Cullen et al. based on the miR-30 precursor and in this case the length and the central bulge found in the native miR-30 is not maintained in the artificial miRNA (native miR-30 precursor is shown in Figure 1 A of Cullen et al.). Figure 5 provides additional examples where Cullen et al. has designed artificial miRNA precursors without regard to maintenance of the precursor secondary structure or miRNA length as compared to the native miRNA precursor, miR-30. In many of the examples in Figure 5, no bulge is present at all, and even when the bulge is present, it is not in the same position nor is it the same size as that found in the native miR-30 precursor.

Nowhere in the text of Cullen et al. is it taught or suggested that maintenance of secondary structure is of concern and, in fact, the contrary is taught. Indeed, no importance is attached to maintenance of the overall structure of the miRNA precursor as is evident from the following passage:

...while the presence of a miR-30 loop may be desirable, variations of that structure can also be tolerated (e.g., loops can be used that are greater than 72%, preferably greater than 79%, more preferably greater than 86%, and most preferably, greater than 93% identical to, for instance, the miR-30 sequence...).

Cullen et al., paragraph 0023.

Accordingly, Cullen et al. teaches a wide variation in the designing of an miRNA precursor. Such variation as suggested by Cullen et al. would be recognized by one of ordinary skill in the art to have the effect of destroying not only the secondary structure but most of the overall structure of the native miRNA precursor.

The Action additionally states that Cullen et al. teaches that the modification of the naturally occurring miRNA precursor "comprises incorporating an miRNA sequence of interest

into said miRNA precursor by substituting stem sequences of its native miRNA to generate miRNAs suitable for use in inhibiting expression of any target gene of interest...." (Action, page 11, emphasis added). However, Cullen et al., in fact, states "the stem sequence in the miR-30 precursor was substituted with a sequence based on the *Drosophila* *nxt* gene." (Cullen et al., paragraph 52). Thus, Cullen et al. teaches substituting the entire miR-30 stem sequence, not just the miRNA sequence found within the stem, as alleged in the Action. Nowhere in Cullen et al. is it stated that only the endogenous miRNA sequence within the stem sequence is replaced by an exogenous miRNA sequence with the sequence opposite the exogenous miRNA sequence in the isolated plant miRNA precursor being modified to maintain the secondary structure of the plant miRNA precursor including double strandedness and any mismatches as is claimed in the present invention.

Further, the resultant miRNA precursor described in paragraph 52 of Cullen et al. is shown in Figure 3A. As discussed previously, this artificial miRNA clearly does not show a native miRNA sequence wherein the endogenous miRNA sequence within the stem is replaced with an exogenous miRNA sequence while maintaining the length of the endogenous miRNA and the secondary structure of the native miRNA precursor as claimed in the present invention. Figure 3A of Cullen et al. shows a designed miRNA that is missing the bulge of the endogenous miRNA and having a length that is different from the endogenous miRNA sequence.

In further support of the contention that Cullen et al. teaches substitution of the entire stem, applicants direct the Examiner's attention to a citation from the Cullen provisional, wherein it states that "[a]dvantageously, the entire miRNA containing stem is complementary to the target sequence, even in regions of the stem 3' and 5' to the miRNA." (Cullen provisional, page 7, lines 7-9). Thus, it is obvious that the Cullen provisional teaches that the entire miRNA-containing stem be replaced, not just the endogenous miRNA sequence, as is taught by the present invention. Clearly, the Cullen provisional upon which Cullen et al. derives its priority date as well as Cullen et al. itself teach away from a miRNA precursor wherein the endogenous miRNA sequence within the stem is replaced with an exogenous miRNA sequence while maintaining the length of the endogenous miRNA and the secondary structure of the native miRNA precursor as claimed in the present invention.

It is unclear why the Action fails to note that Cullen et al. does not take into consideration and, in fact, teaches away from, maintaining the secondary structure of the miRNA precursors. Applicants find this particularly surprising since throughout the prosecution of the present application, the Examiner has repeatedly raised the issue of secondary structure in the designing of artificial miRNAs. For example, in the Office Action mailed October 31, 2006 in the present application, it is stated that "[d]esigning a miRNA precursor comprising a sequence which is complementary to the target sequence of interest would require prediction analysis of said precursors to form an irregular hairpin structure containing various mismatches, internal loops and bulges so that the predicted precursor molecule forms most favorable structure in solution. This involves extensive analysis of secondary structures of the predicted miRNA precursors and selecting the structure with the lowest free energy so that predicted non-natural miRNA precursor is effectively processed...." Cullen et al. mentions the possibility of including bulges but never within the context of the endogenous miRNA precursor secondary structure and as discussed above, in most examples shown in Cullen et al. the artificial miRNA precursors violate one or more aspects of the secondary structure of the native miRNA precursor (see, e.g., Figures 1A, 3A and 5A of Cullen et al.).

Finally, applicants note that the only mention in Cullen et al. of maintaining any of the structure of a naturally occurring miRNA precursor sequence is found in paragraph 0023. However, not only is this paragraph not prior art to the present invention because it is not found in the Cullen provisional but this paragraph still fails to teach or suggest the present invention as it suggests the entire stem sequence be replaced not just the miRNA (similar to Cullen et al. paragraph 52, discussed above)) and then goes on to suggest that variations in loop structure can be tolerated. Cullen et al. simply does not teach or suggest designing miRNA precursors with the features of an endogenous miRNA precursor but with the endogenous miRNA sequence being replaced with an exogenous miRNA sequence wherein the exogenous miRNA sequence maintains the length of the endogenous miRNA sequence and the strand opposite the exogenous miRNA sequence in the isolated plant miRNA precursor is modified to maintain the secondary structure of the plant miRNA precursor including double strandedness and any mismatches as claimed in the present invention.

Further, Llave et al. and Rheinhart et al. fail to remedy the deficiencies of Cullen et al. Llave et al. and Rheinhart et al. discuss native plant miRNA precursors. Neither reference

teaches or suggests a plant stably transformed with an miRNA precursor construct, said miRNA precursor construct comprising a promoter functional in a plant cell, wherein the promoter is operably linked to a nucleotide sequence encoding an isolated plant miRNA precursor, said plant miRNA precursor comprising an exogenous miRNA sequence that replaces an endogenous miRNA sequence and a strand opposite the exogenous miRNA sequence, wherein (a) the exogenous miRNA sequence maintains the length of the endogenous miRNA sequence; and (b) the strand opposite the exogenous miRNA sequence in the isolated plant miRNA precursor is modified to maintain the secondary structure of the plant miRNA precursor including double strandedness and any mismatches, and further wherein the exogenous miRNA sequence is complementary to and hybridizes with a target nucleotide sequence within said plant, whereby the expression of the target sequence is reduced as claimed in the present invention.

The Action alleges that based on the many native miRNA sequences that contain mismatches or bulges seen in Llave et al. and Rheinhart et al., it would have been obvious to maintain the size and positions of mismatches of the native miRNA secondary structure in the non-native miRNA sequence of the modified plant miRNA precursor to avoid any possible problems during processing of the miRNA precursor. (Action, page 13-14). However, the Action provides no basis upon which this conclusion could be made. Nowhere in any cited reference or in the general knowledge of those of ordinary skill in the art at the time this application was filed was the maintenance of the size of the miRNA and the positions of mismatches found in the native miRNA taught or suggested as a way to avoid processing problems or for any other reason. In fact, contrary to the contention of the Action, one of ordinary skill in the art at the time the present application was filed could easily have concluded just the opposite from the disclosures of Llave et al. and Rheinhart et al., i.e., that it was unnecessary to maintain secondary structure because the various different native miRNA precursors of Llave et al. and Rheinhart et al. show variable miRNA sizes and positions of mismatches. Clearly, the skilled artisans in the case of Cullen et al. (filed only weeks prior to the filing of the present invention) made the conclusion that maintenance of secondary structure was unnecessary.

Accordingly, applicants respectfully submit that the present invention is non-obvious in view of Cullen et al., Llave et al. and/or Rheinhart et al. as they fail to teach or suggest all of the

recitations of the claims as required for a rejection under 35 U.S.C. § 103. Therefore, applicants respectfully request the withdrawal of this rejection.

**IV. Rejoinder of claims 1-5.**

Claims 1-5 as presented herein include all of the recitations of product claims 20, 23 and 26. Thus, if it is determined that the products of claims 20, 23 and 26 are allowable, applicants request review and examination of these method claims in the present application, pursuant to the practice of rejoinder as set forth in section 821.04 of the MPEP. In particular, it is stated therein that if a product claim is elected in a restriction and then found allowable, withdrawn process claims that depend from or otherwise include all of the limitations of the allowable product claim are to be rejoined in the same application.

The points and concerns raised in the Action having been addressed in full herein, it is respectfully submitted that this application is in condition for allowance, which action is respectfully requested. Should there be any remaining concerns, the Examiner is encouraged to contact the undersigned attorney by telephone to expedite the prosecution of this application.

No fee is believed to be due with this response. However, the Commissioner is hereby authorized to charge any deficiency or credit any overpayment to Deposit Account No. 50-0220.

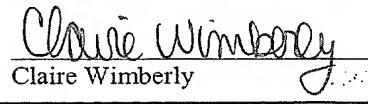
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